TA Instruments Nano ITC protocol

Ian Kleckner
Foster Lab

Equipment

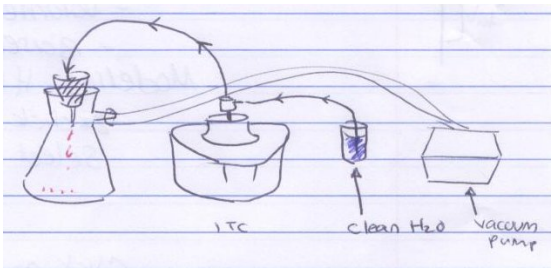
- Sample for cell – 1.2 mL required for 0.950 mL active volume
- Sample for syringe – 300 or 750 µL for 100 or 250 µL desired active volume
  - Note: there are two syringe sizes available, 100 and 250 µL depending on how much ligand you wish you inject
- Buffer – 10+ mL to rinse the cell before loading
- 2 mini stir bars - for degassing both samples

Degassing

- Must actively turn ON each of 4 steps at the degassing instrument
- Set temperature used in experiment
- Set stirring as fast as possible without creating vortex
- Set vacuum to 600 mmHg (maximum 635 mmHg)
- Set timer to 15 min
- Caution: it is possible to evaporate volatile solvent (hence limit to 15 min)

Cleaning

- Rinse with H_2O is typically enough
- Can also wash with 5% Contrad-70 detergent of 5-10% formic acid
- Let this sit in the cell for 20-30 min and then rinse with H_2O
- Cleaning apparatus
  - WATER IN to H_2O source
  - WATER OUT to waste bucket
  - VACUUM to back of degassing station (Menu...vacuum...clean) to turn on



Software (ITC Run)

- Check green light → ITC is on
- To check if experiment is running, look for “Injection #” at top right of screen
- To **stop** experiment
  - Click STOP button
  - **!** Uncheck “Stirrer on”
  - Buret...move up...

Loading cell

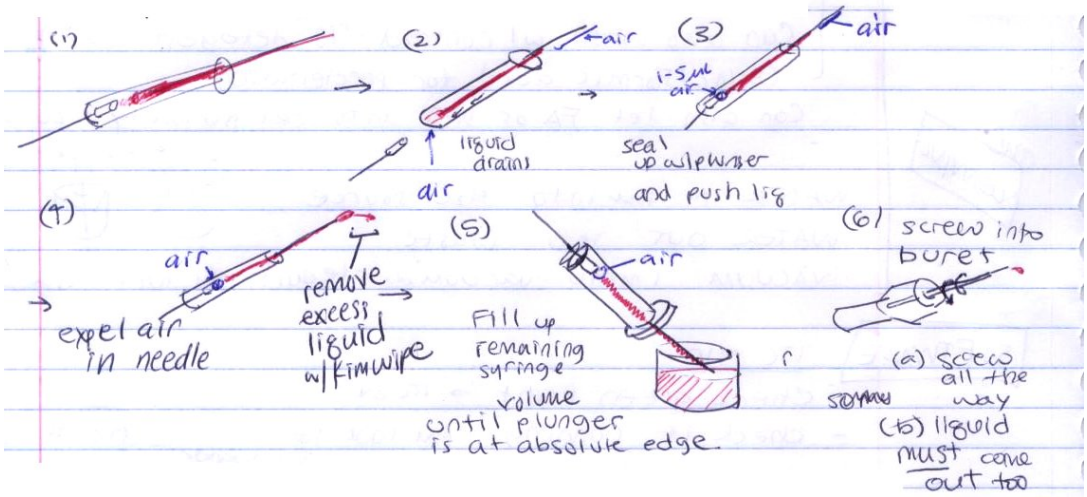
- Rinse cell with buffer 3 times
- Load cell with sample
- It can overflow the cup before inserting the syringe (unlike the VP-ITC), but this is unnecessary

Reference cell

- Fill with degassed water (1.0 mL – do not overfill!)
- The plug and dummy needle can be removed / inserted with tweezers

Loading syringe (ligand)

- Wash with H_2O and/or alcohol to help dry
- Air dry (can use canned air, if it is clean)
- Load sample into syringe
    1. Draw sample from source using the syringe plunger
    2. Remove plunger and allow sample to drain to back of syringe
    3. Cap syringe with plunger to **trap 2-5 µL of air** between plunger tip and sample
    4. Push plunger to expel air in the syringe
    5. Draw sample from source to fill remaining syringe volume (plunger at absolute end of syringe)
    6. Screw into buret and ensure that some sample is driven out by the buret
    7. (Not shown) wipe off excess volume from the tip of syringe with KimWipe



Insert buret into cell

- Insert this into the base in a vertical fashion (do not bend needle!)
- Push down and turn clockwise to engage spring-loaded lock

Software to set up run (ITC Run)

- Check online (green circle)
- Check that stirrer is on
- RPM: 250-500
- Syringe: select 100 or 250 (depending on what was used)
- Setup...Interval 300 sec, 5 µL injection size
- Auto equilibrate: small, medium or large heat expected (default medium)
- Start!

Tips

- User manual is available on PC
- Turn stirring OFF before removing syringe
- Avoid vibration of instrument and table, this will introduce noise into ITC signal
- Do not get electronics wet
- Do not bend the syringe
- Do not drop the buret